CHAPTER VI

Karyotype and Unisexuality of *Leiolepis boehmei* Darevsky & Kupriyanova, 1993 (Sauria: Agamidae) from Southern Thailand

Abstract

A karyological study was carried out for a series of specimens of *Leiolepis boehmei* collected from Nakhon Si Thammarat and Songkhla Provinces in southern Thailand. Mitotic chromosomes were prepared by lymphocyte culturing. A total of 34 diploid chromosomes were found, comprising 12 macrochromosomes and 22 microchromosomes. Secondary constrictions were recognizable on the long arms of the largest metacentric chromosomes. No males were included in the sample consisting of no less than 65 individuals examined. This further confirms the unisexuality of this species assumed by the previous authors. The haploid chromosome arrangement in the karyotype of *L. boehmei* slightly but consistently differs from those of other congeneric species so far karyotyped (including *L. triploida*, a triploid parthenogenetic species) in having fewer microchromosomes (11 vs. 12). This negates the previous assumption of its being one of the parental species of *L. triploida*.

Key words: karyotype, *Leiolepis boehmei*, Agamidae, parthenogenesis, unisexuality

6.1 Introduction

The butterfly lizards of the genus *Leiolepis* Cuvier, 1829 in Southeast Asia include four bisexual *(L. belliana* (Hardwick and Gray, 1827), *L. guttata* Cuvier, 1829, *L. reevesii* (Gray, 1831), and *L. peguensis* Peters, 1971), two unisexual triploid (*L. triploida* Peters, 1971, and *L. guentherpetersi* Darevsky & Kupriyanova, 1993), and one putatively unisexual diploid (*L. boehmei* Darevsky & Kupriyanova, 1993) species. Böhme (1982) argued that a population of *Leiolepis* from Songkhla, southern Thailand, subsequently described by Darevsky and Kupriyanova (1993) as *Leiolepis boehmei*, is distinct from other congeneric species recognized at that date in external morphology and coloration. He also assumed that population to be diploid and parthenogenetic. Furthermore, *L. boehmei* was assumed to have produced *L. triploida*, by means of natural hybridization with males of a widely distributed bisexual species *L. belliana* (Böhme 1982; Darevsky and Kupriyanova 1993). However, the origins of those parthenogenetic species remain unconfirmed (Peters 1971, Böhme 1982, Darevsky and Kupriyanova 1993) and a karyological survey on *L. boehmei* has never been conducted.

In the present study, we karyotyped specimens of this species collected from Nakhon Si Thammarat Province and Songkhla Province, southern Thailand.

6.2 Materials and Methods

Specimens were collected from four localities in southern Thailand: 25 individuals from Hua-Sai District (08°02′N, 100°19′E), Nakhon Si Thammarat

Province, and 25 from Chana District (07°02′N, 100°42′E), 10 from Muang District (07°11′N, 100°16′E) and five from Thepha District (07°54′N, 100°18′E), Songkhla Province (Fig. 6.1), by digging into their burrows found in open sandy habitats. Coloration and morphological characters of specimen captured (Fig. 6.2) matched the description of *L. boehmei* in Darevsky and Kupriyanova (1993). These specimens were also sexed by gonadal investigation through abdominal dissection.

Of these, three individuals from three localities (Hua-Sai District, Chana District, and Thepha District) were karyotyped in the laboratory. Sterile blood samples of individual *L. boehmei* were obtained by cardiac puncture after being anesthetized with ice. Lymphocytes were cultured in supplemented RPMI 1640 media at 37 °C for 72 h. One hour after addition of 0.1 ml colchicine solution (2 mg/ml) per 5 ml culture, cells were harvested, treated with 0.075 mol/l KCl at 37 °C for 30 min, and fixed in Carnoy's solution (1:3, glacial acetic acid: absolute methyl alcohol) two or three times. Finally, metaphase mitotic chromosome preparations were made by an air-dry method and stained in 10 % Giemsa solution for 15 min. To determine the exact chromosome number, 30 metaphase cells were observed from each individual. The karyotype was determined for each individual on the basis of at least 10 well-spread metaphase cells having a complete chromosome set. Terminology for chromosomal description follows Green & Sessions (1991).

All specimens of *L. boehmei* used in this study were preserved in 70% ethanol and deposited in the herpetological collection of the Chulalongkorn University Museum of Zoology (CUB MZ R: see Appendix).

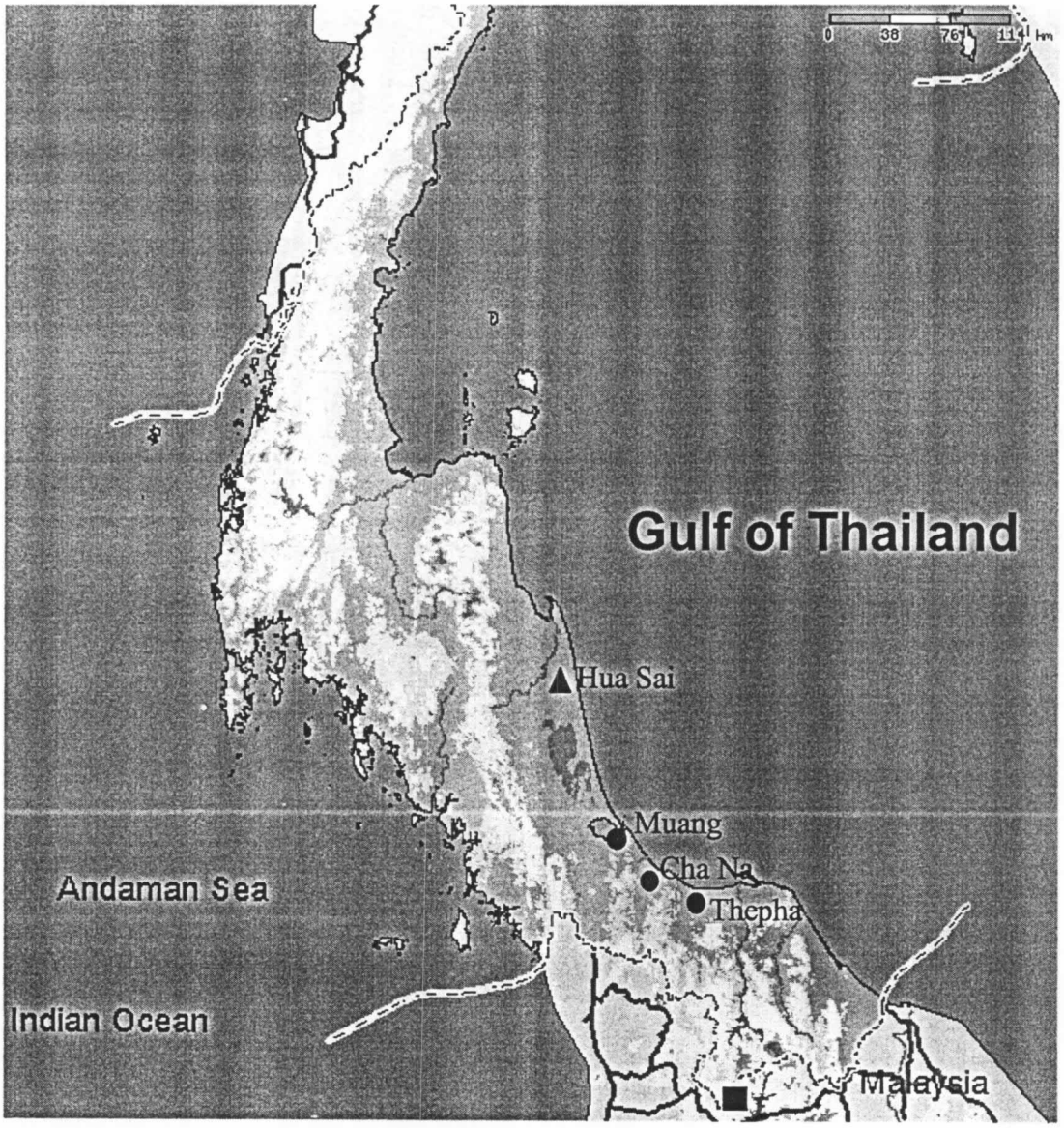

Figure 6. 1 Localities of *Leiolepis boehmei* collected for chromosome examination in this study (▲, Hua Sai District, Nakhon Si Thammarat Province; ●, Muang District, Cha Na District, and Thepha District, Songkhla Province, Thailand) and the locality of *Leiolepis triploida* (■) in northern Malaysia from the study of Darevsky and Kupriyanova (1993).

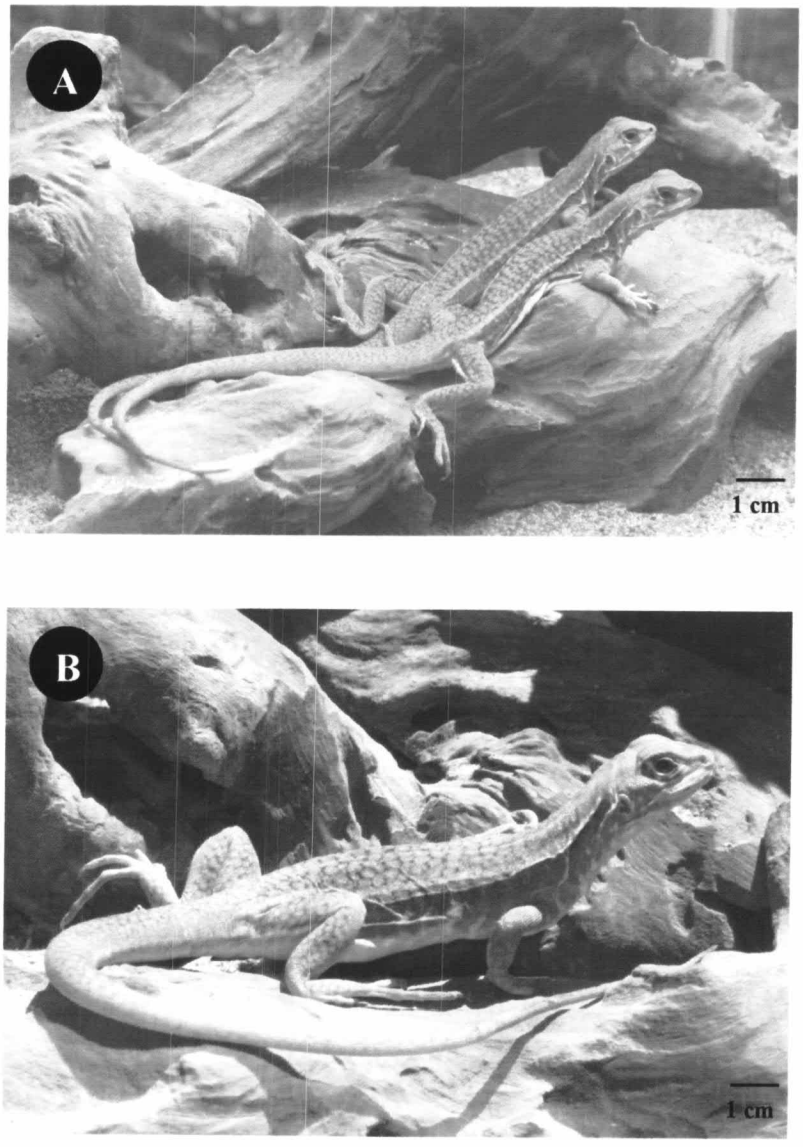

Figure 6.2 Adult female of the unisexual *Leiolepis boehmei* from (A) Hua Sai District, Nakhon Si Thammarat Province, and (B) Cha Na District, Songkhla Province, southern Thailand.

6.3 Results and Discussion

Present specimens, 93-126 mm in snout-vent length (SVL), differ from known species of *Leiolepis* other than *L. boehmei* by having blackish or brownish olive color on upper body with two bold uninterrupted longitudinal stripes of light color that fade solely in the neck region. The ventral surface of the head is gray with white bars and the dorsum of head is olive with a small pale yellowish spot on the lower eyelid. All these and other features exhibited by these specimens were common to *L. boehmei* as described by Darevsky and Kupriyanova (1993). It is therefore obvious that they belong to this species and thus represent substantial northward range extension of the species, because *L. boehmei* has not been recorded from areas north of Songkhla Province (Fig. 6.1). The absence of males in all our samples confirms the unisexuality of *L. boehmei* assumed by previous authors, because in bisexual populations of lizards, males are usually collected more frequently than females, and the complete absence of males is thus considered to be a robust evidence of parthenogenetic reproduction (Darevsky et al. 1988; Peccinini-Seale, 1972).

All specimens karyotyped possessed a standard karyotype of 2n = 34, with one submetacentric and five metacentric pairs of macrochromosomes and eleven pairs of apparently acrocentric microchromosomes. Secondary constrictions were located on the distal portions of long arms of chromosomes no.1 (Fig. 6.3). Its arranged karyotype is shown in Fig. 6.4. The haploid number and morphology of macrochromosomes did not differ from those of other diploid species, *L. belliana, L. guttata* and *L. reevesii* (Kupriyanova, 1984; Shoubai et al., 1987; Solleder & Schmid 1988), and of the triploid *L. triploida* (Hall, 1970). Even so, however, the haploid

karyotype of *L. boehmei* differs from those of other congeneric species in possessing fewer microchromosomes (11 vs. 12). No sex chromosome heteromorphisms were evident in the karyotype of *L. boehmei* like in karyotypes of other *Leiolepis* species.

It is possible that the 2n = 34 karyotype of this unisexual lizard has reduced the microchromosome number by Robertsonian fusion, or deletion of chromatin (Witten, 1983). There may be two possible processes for such a reduction of microchromosomes: at stage of parental species (if the parthenogenetic form in problem is of hybrid origin), or after establishment of a clonal lineage (Ota et al., 1989).

Based on nine female *L. boehmei* samples from Songkhla, Böhme (1982) proposed that *L. triploida* might be an allotriploid hybrid clone between the diploid- parthenogenetic females and males of the normal bisexual species *L. belliana*. In support of Böhme's hypothesis, Darevsky and Kupriyanova (1993) noted that some character states in coloration and external morphology of *L. triploida* are intermediate between those of *L. boehmei* and *L. belliana*. However, the differential haploid number of microchromosomes in *L. boehmei* (11) does not support this hypothesis. Intensive surveys in Songkhla and nearby provinces did not find any individual *L. triploida*. In addition, no other unisexual species of *Leiolepis* have been reported from Malaysia or southern Thailand. It is therefore probable that *L. triploida* was originated through autopolyploidy as a consequence of spontaneous reorganization of the originally diploid karyotypes (Peters 1971: see Witten (1978) for an example of autotriploidy in the Agamidae). Further studies using techniques of allozymic and mitochondrial sequence variation analyses, such as those employed by Schmitz et al.

(2001), are desired to establish a convincing hypothesis for the origins of *L. boehmei* and *L. triploida*.

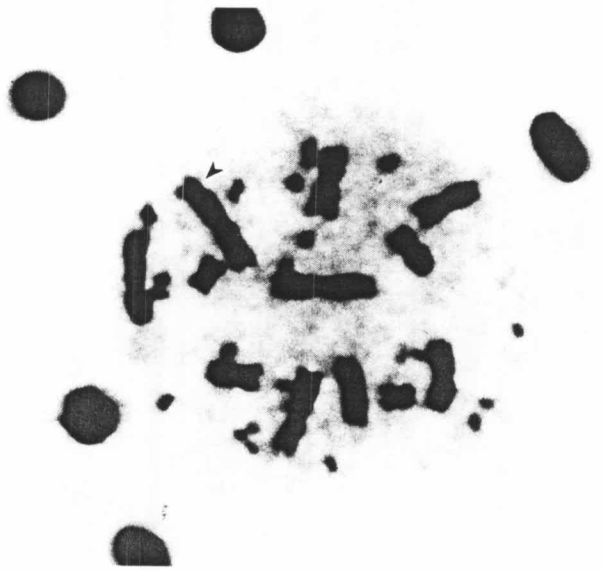

Figure 6.3 Mitotic metaphase chromosome of *Leiolepis boehmei*. An arrow indicates a secondary constriction on the long arm of macrochromosome no. 1.

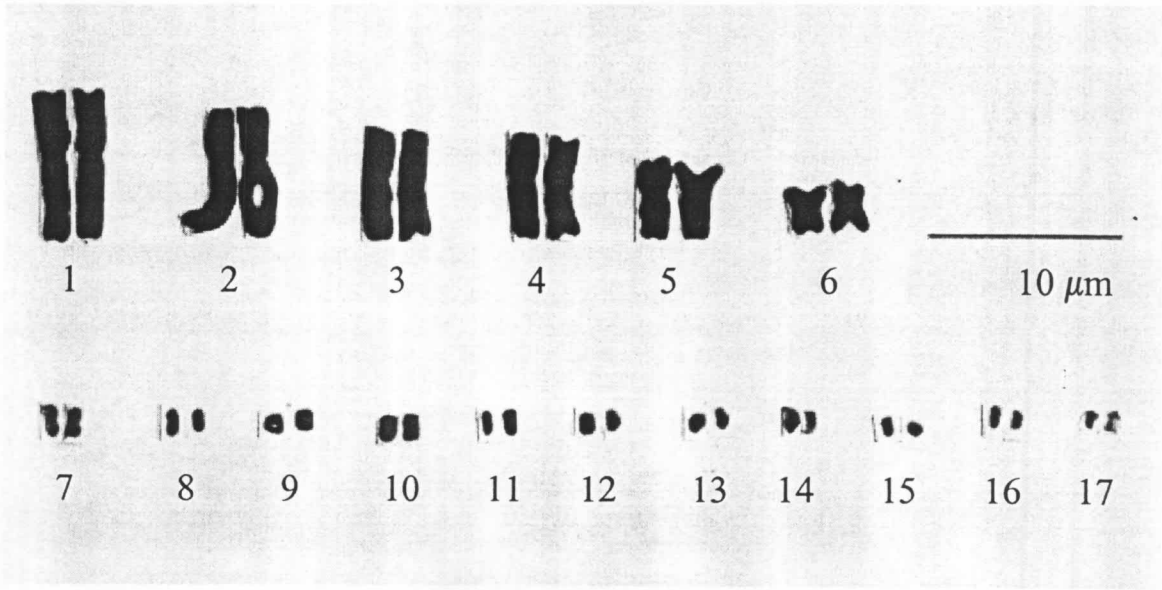

Figure 6.4 Karyotype of *Leiolepis boehmei*, 2n = 34 with 12 macrochromosomes and 22 microchromosomes.

Acknowledgements

We are very grateful to Professor Hidetoshi Ota and Dr. Kanok Lertpanich for his valuable comments and suggestions. Special thanks to Jarujin Nabhitabhata, Tanya Chan-ard, Noppadon Kittana, and Jonathan Murray for their help and providing information. We would like to thank Sumate Aranyavalai for field assistance. This work was financially supported by the Ministry of University Affairs, the Ministry of Education, and the Graduate School of Chulalongkorn University.

APPENDIX

Voucher specimens of *Leiolepis boehmei* used in this study. CUB MZ R 2004.1-25, Hua Sai, Nakhon Si Thammarat; , CUB MZ R 2004.26-50, Cha Na, Songkhla; CUB MZ R 2004.51-60, Muang, Songkhla; CUB MZ R 2004.61-65, Thepha, Songkhla.